IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:	)	
	)	Before the Examiner
BARBER, et al.	)	Elli Peselev
	)	
Serial No. 10/018,467	)	Group Art
	)	Unit 1623
Filed April 4, 2002	)	
	)	
ANTIBIOTIC AGENTS	)	

DECLARATION OF JILL BARBER
SUBMITTED UNDER 35 USC 6131

I, Jill Barber, a British subject of 22 Arthog Road, Didsbury, Manchester, M20 6HG, Great Britain, declare as follows.

1. I am making this Declaration in support of US Patent Application No. 10/018,467 (herein referred to as "the Application").

2. I am named as a co-inventor in the Application.

3. I was awarded the degrees B.A., M.A. and Ph.D by the University of Cambridge and am a member of the Royal Society of Chemistry and Chartered Chemist. I have been employed by The Victoria University of Manchester, Oxford Road, Manchester, M13 9PL, Manchester, Great Britain as a Senior Lecturer since 1993 specialising in Medicinal Chemistry.

4. I have reviewed and am familiar with the Application.

5. Tests were conducted under my direction to establish the acid stability of the following esters:

(i) Erythromycin B 2'-ethyl succinate (EBES), and
(ii) Erythromycin A 2'-ethyl succinate (EAES)

over a range of pH conditions that are likely to be encountered in the stomach of a human patient.

6. The compounds tested for acid degradation were dissolved in deuterated Britton-Robinson buffer at apparent pH 2.0 or in deuterated phosphate buffer at apparent pH 2.01 to give concentrations of either 4mM or 2mM, depending on the solubility of the compound. The degradation experiments were performed by acquiring 1D-^{1}H spectrum (using a PRESAT pulse – presaturation for water suppression) at regular intervals. All degradation studies were monitored at 37° C. TSP, at a concentration of 1mM, was used as a reference standard. The proton peaks to be monitored were selected and normalised against

TSP. After baseline correction, the integral values of the selected peaks were measured over time. The spectra were processed with reference deconvolution using the FIDDLE algorithm (Barjat *et al*., J. Mag. Res. Series A 1995, 116, 206-214). This algorithm compares the experimental time-domain signal of a reference with that predicted by theory, multiplies the raw experimental data by the complex ratio of the two signals to produce the corrected free induction decay (FID).

7. The results of these tests detailed in paragraph 6 are shown in Table 1 which gives the half – life in minutes of EBES and EAES at various pH values tested.

Table 1

Half-lives (mins) EBES and EAES at acidic pH

	Apparent pH (pD ≡ apparent pH + 0.4)				
	2	2.5	3	3.5	4
EBES	ca 70	> 180	> 420	n/t	n/t
EAES	< 5	< 5	< 10	58	170

n/t = "not tested".

8. The results in Table 1 clearly demonstrate the enhanced stability of EBES as compared to EAES over a range of acid pH conditions. As shown in Table 1, EBES was stable for ca 70 mins (1h 10min) at pH2, greater than 180 mins (3h) at pH 2.5, and greater than 420 mins (7hr) at pH 3. In view of trend, stability of EBES at pH values of 3.5 and 4 were not tested.

9. Tests were also conducted under my direction to provide a comparative degradation study of Erythromycin B 2'-ethyl succinate (EBES), Erythromycin A 2'-ethyl succinate (EAES) and Erythromycin B enol ether 2'-ethyl succinate (EBEES) under conditions mimicking a paediatric formulation and storage thereof. In order to determine the effect of pH and solubility on the extent of hydrolysis, the compounds were formulated as suspensions in water (25 mg ml^{-1}) and were stored in a refrigerator at 4°C for 21 days. Subsequently the samples were extracted with chloroform and one dimensional ^1H spectra were acquired. Signals at approximately 3.20 (H-2' in the free drug) and 4.75 (H-2' in the ester) were among those used to determine the extent of hydrolysis. The results are shown in Table 2.

Table 2

Drug	Extent of hydrolysis to erythromycin A or erythromycin B (%)	
	pH 6.0	pH 8.0
EBES	68.2	23.1
EBEES	0.0	0.0
EAES	26.2	30.1

Table 2 clearly demonstrates the storage stability of the 2'-ester of Erythromycin B enol ether.

10. The improved stability of EBES relative to EAES as demonstrated in Table 1 can be expected to be shared for all 2'- Erythromycin B (EB) esters as compared to the corresponding esters of Erythromycin A (EA). This is due to the fact that the EA esters bear a hydroxyl group at C12 whereas the EB esters do not. Exactly the same argument applies to esters of Erythromycin B enol ether (EBEE) relative to the corresponding esters of Erythromycin A enol ether (EAEE) and this is clearly demonstrated by the data presented in Table 2.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the Application or any patent issuing thereon.

24th June 2004
Date

Jill Barber
Signature

Typed Name Jill Barber

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

BARBER et al — Atty. Ref.: 39-252; Confirmation No. 8122

Appl. No. 10/018,467 — TC/A.U. 1623

Filed: April 4, 2002 — Examiner: Peselev, E.

For: ANTIBIOTIC AGENTS

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION

We, Jill Barber and Mohammed N. Mordi, do hereby declare and state as follows.

1. We are co-inventors of the subject matter claimed in the above application.

2. We are co-authors, together with Gareth A. Morris, Michelle D. Pelta and Valerie Boote of an article entitled: "Acid-Catalyzed Degradation of Clarithromycin and Erythromycin B: A Comparative Study Using NMR Spectroscopy" published in the Journal of Medicinal Chemistry 43:467-474 (2000) (hereinafter "the Article").

3. To the extent that the Article discloses or suggests the invention claimed in the above-identified application, that invention is our own. While Gareth Morris, Michelle Pelta and Valerie Boote made contributions to the subject matter of the Article

that warranted their being named as co-authors, these individuals did not contribute to the conception of the invention claimed in the above-identified application, as will be clear from the comments that follow:

Gareth Morris is an NMR expert in the Department of Chemistry at the University of Manchester (UMAN). Professor Morris made significant contributions to the Article in that he designed the NMR experiments described and deduced the kinetic parameters. Professor Morris was responsible for several novel experiments conducted during the course of the studies reported in the Article.

Michelle Pelta was Professor Morris' graduate student in the Department of Chemistry at UMAN. Ms. Pelta (now Dr. Pelta) contributed to the results reported in the Article in the context of the Diffusion Ordered SpectroscopY (DOSY) time course and associated automated shimming.

Valerie Boote is a senior technician in the Department of Chemistry at UMAN. She made significant technical contributions to the mass spectrometric data reported in the Article.

4. As will be clear from the details presented in paragraph 3 above, co-authors Morris, Pelta and Boote made significant contributions to the studies reported in the Article, however, they did not contribute to the conception of the invention claimed in the above-identified application.

We hereby declare that all statements made herein of our own knowledge are true and that statements made on information and belief are believed to be true; and further

that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any parent issued thereon.

Further, declarants say not.

Jill Barber

24th June 2004
Date

Jill Barber

Mohammed N. Mordi

29th June 2004
Date

TOTAL P.03

TOTAL P.04